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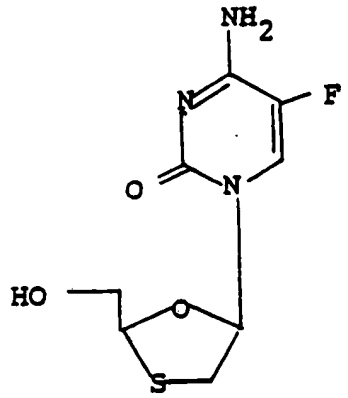

(57) Abstract

The use of a 1,3-oxathiolane nucleoside analogue and pharmaceutically acceptable derivatives thereof for the treatment of hepatitis B virus infections is disclosed. Pharmaceutical formulations are also provided.

USE OF 5-FLUORO-2'-DEOXY-3'-THIACYTIDINE FOR THE TREATMENT OF HEPATITIS B

The present invention relates to the use of a 1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-cytosine derivative and physiologically functional derivatives thereof for the treatment of hepatitis B viral infections.

Hepatitis B virus (HBV) is a viral pathogen of major worldwide importance. HBV is most common in Asian Countries, and prevalent in sub-Saharan Africa. The virus is etiologically associated with primary hepatocellular carcinoma and is thought to cause 80% of the world's liver cancer. In the United States more than ten thousand people are hospitalized for HBV illness each year, an average of 250 die with fulminant disease. The United States currently contains an estimated pool of 500,000 - 1 million infectious carriers. Chronic active hepatitis will develop in over 25% of carriers and often progresses to cirrhosis. It is estimated that 5000 people die from HBV-related cirrhosis each year in the U.S.A. and that perhaps 1000 die from HBV-related liver cancer. Even when a universal HBV vaccine is in place, the need for effective anti-HBV compounds will continue. The large reservoir of persistently infected carriers, estimated at 220 million worldwide, will receive no benefit from vaccination and will continue at high risk for HBV-induced liver disease. This carrier population serves as the source of infection of susceptible individuals perpetuating the instance of disease particularly in endemic areas or high risk groups such as i.v. drug abusers and homosexuals. Thus, there is a great need for effective antiviral agents, both to control the chronic infection and reduce progression to hepatocellular carcinoma.

Clinical effects of infection with HBV range from headache, fever, malaise, nausea, vomiting, anorexia and abdominal pains. Replication of the virus is usually controlled by the immune response, with a course of recovery lasting weeks or months in humans, but infection may be more severe leading to persistent chronic liver disease as

thereof for use in the treatment or prophylaxis of a hepatitis B virus infection. According to a further feature of the present invention we provide the use of the compound of formula (I) or a physiologically functional derivative thereof, in the manufacture of a medicament for the treatment or prophylaxis of a hepatitis B virus infection.
In a further aspect of the present invention there is included a method for the treatment or prophylaxis of a hepatitis B virus infection in a host, for example, a mammal such as a human which comprises treating the host with a therapeutically effective amount of the compound of formula (I) or a physiologically functional derivative thereof.

By "physiologically functional derivative" is meant a pharmaceutically acceptable salt, amide, ester or salt of an ester of the compound of formula (I) or any other compound which upon administration to the recipient, is capable of providing (directly or indirectly) the said compound of formula (I) or an active metabolite or residue thereof.

Preferred esters in accordance with the invention include carboxylic acid esters in which the non-carbonyl moiety of the carboxylic acid portion of the ester grouping is selected from straight or branched chain alkyl e.g. n-propyl, t-butyl, n-butyl, alkoxyalkyl (e.g. methoxymethyl), arylalkyl (e.g. benzyl), aryloxyalkyl (e.g. phenoxymethyl), and aryl (e.g. phenyl); sulfonate esters such as alkyl- or arylalkylsulfonyl (e.g. methanesulfonyl); amino acid esters (e.g. L-valyl or L-isoleucyl); dicarboxylic acid esters (e.g. hemisuccinate); and 5'- mono- di- or tri-phosphate esters. The phosphate esters may be further esterified by, for example, a C_{1-20} alcohol or reactive derivative thereof, or by a 2,3-di(C_{6-24})acyl glycerol. Any alkyl moiety present in such esters advantageously contains 1 to 18 carbon atoms, particularly 1 to 4 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group optionally substituted e.g. by halogen, C_{1-4} alkyl, C_{1-4} alkoxy or nitro.

about 75μM, preferably about 2 to 50μM, most preferably about 3 to about 30μM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1 to about 100 mg/kg of the active ingredient. Desirable blood levels may be maintained by a continuous infusion to provide about 0.01 to about 5.0 mg/kg/hour or by intermittent infusions containing about 0.4 to about 15 mg/kg of the active ingredient.

In the manufacture of a medicament according to the invention, hereinafter referred to as a "formulation", the compound of formula (I) or a physiologically functional derivative thereof herein as "active ingredient", is typically admixed with, inter alia, one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents.

The formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injections solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, as liposomes or other microparticulate systems which are designed to target the compounds to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

The compound of formula I may be prepared for example by:

a) reacting an optionally protected 5-F-cytosine compound with a 1,3-oxathiolane of formula IIA

(wherein R_1 is as defined above) with an agent serving to convert the oxo group in the 4-position of the uracil ring to an amino group; any remaining protecting groups being removed for example by acid or base hydrolysis to produce the desired product.

With regard to process a), the hydroxy protecting group includes protecting groups such as acyl (e.g. acetyl), arylacyl (e.g. benzoyl or substituted benzoyl), trityl or monomethoxytrityl, benzyl or substituted benzyl, trialkylsilyl (e.g. dimethyl-t-butylsilyl) or diphenylmethylsilyl. The 5-R-cytosine compound may be optionally protected with silyl, e.g. trimethyl silyl groups. Such groups may be removed in conventional manner. The leaving group L is a leaving group typical of those known in the art of nucleoside chemistry e.g. halogen such as chlorine or bromine, alkoxy such as methoxy or ethoxy or acyl such as acetyl or benzoyl.

The reaction in process a) may be effected in an organic solvent (e.g. 1,2-dichloroethane or acetonitrile) in the presence of a Lewis acid such as stannic chloride or trimethylsilyl triflate.

Compounds of formula IIA may be obtained from a suitably protected 2-hydroxyacetaldehyde of formula III,

$$R_1OCH_2CHO \qquad (III)$$

wherein R_1 is defined above, as described in Can. J. Research, 8, 129 (1933) and European Patent Specification 0 382 526. Reaction of compounds of formula III with a mercaptoacetal $HSCH_2CH(OR)_2$, wherein R is C_{1-4} alkoxy such as $HSCH_2CH(OC_2H_5)_2$, known in the art (Chem. Ber. 85:924-932, 1952), yields compounds of formula IIA wherein L is OR (alkoxy) e.g. methoxy or ethoxy. Alternatively, compounds of formula IIA, wherein L is alkoxy, may be converted to compounds of formula IIA wherein L is halogen or acyl by methods known in the art of carbohydrate chemistry.

The compound of formula (I) may be converted into a pharmaceutically acceptable esters and amides by reaction with an appropriate acylating agent, for example, an acid halide or anhydride serving to acylate the 5'-OH and 4-NH_2 groups. The acyl group may then be removed selectively from one or other of the 5'-OH and 4-NH_2 groups. For, example, treatment of the diacylated compound under acidic conditions, eg. a Lewis acid such as zinc bromide in methanol, removes the 4N-acyl group to yield the corresponding 5'-OH ester where treatment of the diacylated compound under alkaline conditions, eg. with sodium methoxide removes the 5'-OH acyl group to yield the corresponding 4N-amide. The acyl groups can also be removed selectively by treatment with commercially avialable esterase or lipase enzymes, eg. pig liver esterase or pancreatic lipase, or by treatment in accordance with methods described in U.S. Patent Specification No. 5071983. The compound of formula (I) may be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by

W.L. Sung, Nucleic Acids Res. 9:6139, 1981, using 1,2,4-triazole and 2 equivalents of 4-chlorophenyldichlorophosphate in dry pyridine at ambient temperature. This conversion is followed by reaction with methanol previously saturated with ammonia at 0°C, and the 2-acetate is hydrolyzed to give (±)-cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine.

Pharmaceutical Formulations

In the following formulation Examples, the "Active Ingredient" is cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine.

Example 2

Tablet Formulations

The following formulations A, B and C are prepared by wet granulation of the ingredients with a solution of povidone, followed by addition of magnesium stearate and compression.

Formulation A

	mg/tablet	mg/tablet
(a) Active ingredient	250	250
(b) Lactose B.P.	210	26
(c) Povidone B.P.	15	9
(d) Sodium Starch Glycollate	20	12
(e) Magnesium Stearate	5	3
	500	300

Formulation E

	mg/tablet
Active ingredient	250
Lactose	150
Avicel	100
	500

Formulation F (Controlled Release Formulation)

The formulation is prepared by wet granulation of the ingredients (below) with a solution of povidone followed by the addition of magnesium stearate and compression.

		mg/tablet
(a)	Active ingredient	500
(b)	Hydroxypropylmethylcellulose (Methocel K4M Premium)	112
(c)	Lactose B.P.	53
(d)	Povidone B.P.	28
(e)	Magnesium Stearate	7
		700

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Example 3

Capsule Formulations

Formulation A

A capsule formulation is prepared by admixing the ingredients of Formulation D in Example 2 above and filling into a two-part hard

spheronization of the extrudate and drying. The dried pellets are then coated with controlled-release membrane (d) and filled into a two-piece, hard gelatin capsule.

		mg/capsule
(a)	Active ingredient	250
(b)	Microcrystalline Cellulose	125
(c)	Lactose B.P.	125
(d)	Ethyl Cellulose	13
		513

Example 4

Injectable Formulation

Formulation A

Active ingredient	0.200 g
Hydrochloric acid solution, 0.1 M, or Sodium hydroxide solution, 0.1 M q.s. to pH	4.0 to 7.0
Sterile water	q.s. to 10 mL

The active ingredient is dissolved in most of the water (35°C-40°C) and the pH adjusted to between 4.0 and 7.0 with the hydrochloric acid or the sodium hydroxide as appropriate. The batch is then made up to volume with the water and filtered through a sterile micropore filter into a sterile 10 mL amber glass vial (type 1) and sealed with sterile closures and overseals.

Formulation B

Active ingredient	0.125
Sterile, pyrogen-free, pH 7 phosphate Buffer,	q.s. to 25 mL

One-fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45°C maximum. The active ingredient is sifted through a 200 M sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until smooth dispersion is achieved. Maintaining the mixture at 45°C, the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 M stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C, 2.02 g of the mixture is filled into suitable, 2 mL plastic molds. The suppositories are allowed to cool to room temperature.

Example 8

Pessaries

	mg/pessary
Active ingredient	250
Anhydrate Dextrose	380
Potato Starch	363
Magnesium Stearate	7
	1000

The above ingredients are mixed directly and pessaries prepared by direct compression of the resulting mixture.

Example 9

Antiviral Activity Against Hepatitis B Virus (HBV)

The compound cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluoro-cytosine, was tested as described below.

The human HBV producer cell line of $HepG_2$, 2.2.15, described and characterized by Sells et al., PNAS 84:1005, 1987 and J. Virol. 62:2836, 1988, has been shown to share many characteristics of the HBV chronically infected hepatocyte. It is infectious as demonstrated by

Effect of cis-1-(2-(Hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine on HBV Production in 2.2.15 Cell Cultures

Intracellular HBV DNA*

Treatment Compound (μm)	Integrated	Monomer-	Replicative intermediate	HBV DNA in Culture Medium (pg/mL)++ Day 0	Day 3	Day 6	Day 10
A. untreated cells	1.1	2.0	81	58	67	93	77
	0.9	2.3	77	89	110	100	88
100	1.9	0.8	2	64	11	3	0
	1.5	1.9	1	34	19	2	0
B. untreated cells	1.5	1.9	110	65	44	86	71
	1.0	2.3	67	90	120	80	82
100	1.6	0.8	1	90	16	0	0
	1.0	0.7	1	74	10	0	0

* Analysis of intracellular HBV DNA (Dane particles) was 24 hours following the 10th day of treatment.

++ A "zero" indicates an undetectable level of HBV DNA, sensitivity cutoff was 0.1pg/mL

7. A compound of formula (I) (as defined in claim 1) or a physiologically functional derivative thereof for use in the treatment or prophylaxis of a hepatitis B virus infection.

8. Cis-1-(2-(Hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine for use in the treatment or prophylaxis of a hepatitis B virus infection.

9. A method of treating a human having a hepatitis B virus infection comprising the administration to said human of an effective anti-hepatitis B treatment amount of a compound of formula (I) (as defined in claim 1) or a physiologically functional derivative thereof to said human.

10. A method as claimed in claim 11 in which the said compound of formula (I) is cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	Gastroenterology, vol. 94, no. 5, part 2, 1988, C. KASSIANIDES et al.: "Effects of 2',3'-dideoxycytidine on duck hepatitis B virus", page A552, see the abstract	1-6,9, 10